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PHYTOPATHOLOGY

VOLUME X NUMBER 9

SEPTEMBER, 1920

RELATION OF CLIMATE TO THE DEVELOPMENT AND CONTROL OF LEAF ROLL OF POTATO

PAUL A. MURPHY AND E. J. WORTLEY

WITH ONE FIGURE IN THE TEXT

Prior to the pioneer work of Quanjer and Oortwijn Botjes (2) in Europe and that of the authors (1) in America, in which the infectious nature of leaf roll was proved, there was a tendency to over-emphasize the influence of environment on the disease. It was held that the presence of leaf roll in hot, dry regions was in some way attributable to the climate, leaving it a difficult question to account for the occurrence of the disease in parts of Canada in which the seasons are comparatively cool and moist. The prevalence of neighbor-infection at Charlottetown, Prince Edward Island, (Murphy and Wortley, loc. cit.) furnished a satisfactory solution of the latter difficulty, it being shown that once leaf roll seed is introduced the spreading-power of the disease is such as to account for its persistence and even for its increase from year to year under the favorable conditions of Prince Edward Island. There remained to be explained why it is that leaf roll is a factor of so much greater economic importance in warmer districts than in the cool climate of the Maritime Provinces.

RESULT OF PLANTING SISTER TUBERS IN PRINCE EDWARD ISLAND, BERMUDA AND SOUTHERN ONTARIO

An experiment was begun in 1917 to test the effect of the climate of Prince Edward Island, Bermuda and Southern Ontario on the development of leaf roll. A crop of Garnet Chili potatoes which had a considerable percentage of leaf roll was selected in Nova Scotia in 1916, and 238 individual plants were dug which yielded three or more tubers. The tubers from each hill were given the same number and one of each was sent to Charlottetown, Bermuda and Ottawa, where they were planted in tuber units. The results are shown in table 1. As there was

considerable loss from rot and other causes, about half the hills were not represented at Ottawa, but there was practically a full muster in Charlottetown and Bermuda.

This result is rather striking, emphasizing as it does the importance of the seed as a carrier of leaf roll. Comparing Bermuda, which is very liable to the disease, and Prince Edward Island, which is naturally free from it, it is found that only 14 plants out of 222 failed to react similarly at both places. Five of these were leaf roll at Charlottetown and healthy in Bermuda, while 9 were healthy at Charlottetown and leaf roll in Bermuda. It is not believed that there was any difference in diagnosis, nor is it likely that the plants became infected locally and showed the disease the same year. It is probable that the difference existed in the parent hill because it is not infrequent to find healthy and affected sister tubers in a diseased crop. Thus it appears from this experiment that where leaf roll appears it is to be traced almost altogether if not entirely so to infected seed and not directly to climatic conditions.

TABLE 1

Result of planting sister tubers from a partly diseased crop in Prince Edward Island, Bermuda and Southern Ontario

	NUMBER OF PLANTS	CASES IN WHICH PLANTS REACTED SIMILARLY AT ALL PLACES	
		Number	Per cent
Plants represented in three places.....	102	93	91.2
Plants represented in two places.....	222	208	93.7

It is interesting to follow these two lots of potatoes for another year. The Bermuda crop after being harvested about May, 1917, was placed in cold storage, it being then too late for the Canadian season of that year, but it was sent to Charlottetown and planted there in 1918 near the corresponding Charlottetown seed. The latter was re-planted in a new plot hill by hill in the same order in which it came before. It was then found that 60.7 per cent of the Charlottetown seed was affected with leaf roll while 97.5 per cent of the Bermuda seed was diseased. The percentage of leaf roll in the Charlottetown lot the previous year had been 19 and the Bermuda lot was practically the same, there being only four more leaf roll plants found there than in Prince Edward Island. The subsequent difference must have arisen from infection being more successful under Bermuda conditions, and it is here that the influence of climate is seen, as a secondary factor which predisposes to the disease.

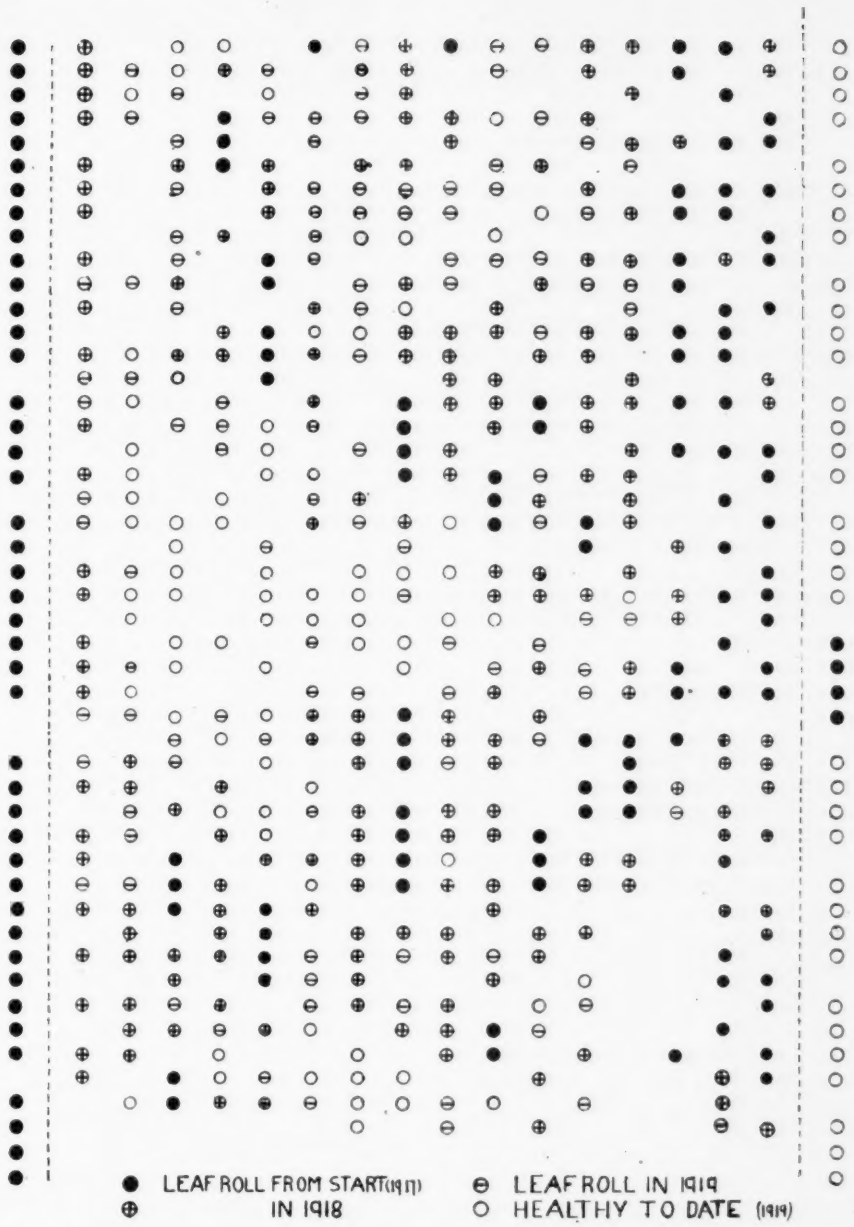

FIG. 1. THE CHARLOTTETOWN PLOT (AS IT STOOD FOR THREE YEARS) OF THE EXPERIMENT IN WHICH SISTER TUBERS WERE PLANTED IN PRINCE EDWARD ISLAND, SOUTHERN ONTARIO AND BERMUDA

The circles inside the dotted lines represent plants, each of which was put back in its own place (but in a new plot) in 1917, 1918, and 1919. The plants outside the lines were present in 1917 only, as they belonged to another experiment. They are shown because they infected the outside rows particularly to the right. The spread of leaf roll from the original infection centers can be followed. The percentages of leaf roll were: 1917, 19 per cent; 1918, 60.7 per cent; 1919, 85.6 per cent.

NEIGHBOR-INFECTION IN VARIOUS PARTS OF CANADA

These conclusions are borne out by a later experiment of the first-named author in which it was found that the spread of the disease varied greatly in different parts of Canada. Uniform seed of the variety Empire State, both healthy and diseased, was planted in the nine localities listed in table 2. The plots were so arranged that there were two rows of leaf roll plants with one row of healthy plants between them, and five rows of healthy plants outside them on both sides, thus:

H H H H H LR H LR H H H H H

Nothing in particular happened during the first year, all the plants from the healthy and leaf roll seed being healthy and leaf roll respectively.

TABLE 2

Spread of leaf roll from diseased to healthy plants in various parts of Canada (uniform Empire State seed throughout)

LOCALITY	PER CENT LEAF ROLL PRESENT IN HEALTHY ROWS					
	Between two rows of leaf roll	Next row to leaf roll	Second row from leaf roll	Third row from leaf roll	Fourth row from leaf roll	Fifth row from leaf roll
Charlottetown, P. E. I.	15.0	21.2	2.5	2.5	0	0
Kentville, N. S.	35.0	12.5	6.5	1.2	2.5	1.2
Nappan, N. S.	20.0	16.2	0	0	0	0
Fredericton, N. B.	5.0	0	3.7	1.2	1.2	0
Lennoxville, P. Q.	17.5	17.5	0	0	0	3.7
Ottawa, Ont.	72.5	50.0	23.7	22.5	13.7	10.0
Thunder Bay, Ont.	0	0	0	0	0	0
Brandon, Man.	13.8	5.6	12.3	3.2	7.2	8.0
Indian Head, Sask.	5.3	1.4	1.4	0	0	0

Seed was saved separately from each row and planted in an experiment of the same plan in 1919. A sample from each row from most of the experiments was also grown at Charlottetown. Leaf roll appeared in the second year in varying amounts in the healthy rows (table 2, where each pair of corresponding rows is averaged), there being in general most in the center row and progressively smaller amounts in the outer ones.

It is probable that practically no dispersal took place at Brandon, Man., Indian Head, Sask., or Fredericton, N. B., in spite of the figures recorded. The inspection at these places was made under difficulties, and the corresponding samples showed no disease except 0.5 per cent at Fredericton. As to the other experiments, no infection took place at Thunder Bay in Northern Ontario, a considerable amount occurred at Charlottetown, P. E. I., Lennoxville, P. Q., and Nappan, N. S., still

more at Kentville, N. S., and at Ottawa, Ont., more than twice as much as at any other place.

No entirely adequate reason can be given for these variations, although it may be significant that leaf roll is found more or less commonly in the districts around Ottawa and Kentville. There are indications that the dispersal of leaf roll varies not only from place to place but from season to season in the same locality. While at Charlottetown it has occurred regularly for three seasons, the percentage of infection contracted by healthy Garnet Chili potatoes grown between two rows of leaf roll plants being 89.5, 62.4, and 50 per cent respectively, a preliminary attempt to bring about infection in the same variety at Kentville, Nova Scotia, in 1917–1918 resulted in comparative failure, only 3 per cent of the plants becoming diseased. Nevertheless the extent to which plants are in danger from neighboring diseased ones, if only in certain years at some places, is sufficient to account for the persistence of the disease and for failure to eradicate it by the commonly recommended methods of hill selection.

NEIGHBOR-INFECTION AND CONTROL BY MEANS OF SELECTION

When work was first begun jointly in 1915 in the affected area of Nova Scotia it was believed that leaf roll should in time eliminate itself since diseased plants produce only about one-third of a crop. The then general view was also held that the selection of the largest and healthiest hills from a partially diseased crop would hasten this process materially. Experiment and observation proved both of these views erroneous before the underlying reason, the infectious nature of the disease, was discovered.

A scheme of seed selection on a large scale was begun in coöperation with the Garnet Chili growers in King's County, Nova Scotia, where leaf roll was very common and was jeopardizing the seed trade with Bermuda. Things had come to such a pass that the Bermuda government had arranged for the transfer of some of the best stock to Maine with the purpose of drawing its supply from there. The Nova Scotia growers were thoroughly alarmed and willingly undertook any method that promised a way out. In the Church Street district thirteen of the growers with the best seed selected by hand from one-half barrel to fifteen barrels of seed from the best plants in their fields and planted it in 1916 beside seed from the general crop. The result was so far from expectation that it was difficult to explain it on the old theory of the nature of leaf roll, for there was on the average practically as much of the disease in the selected as the unselected seed. For example one grower had 41 per cent of leaf roll in his seed plot while another had 25 per cent.

Although proof was thus given for the first time that the selection of large plants from a crop affected with leaf roll does not eliminate the disease, a further attempt was made in 1916 to improve large areas of seed by rogueing it in the field before it was shipped to Bermuda. The result was again the same, for a high percentage of leaf roll developed in these stocks when they were planted in the South. It was just after these events that the paper of Quanjer et al. (loc. cit.) was received, in which the infectious nature of leaf roll seemed to be proved. Further proof was actually at that time in our hands, for one of the seed-plots just mentioned which had 25 per cent of leaf roll in 1916 showed 40 per cent in 1917. The more exact experiments above recorded definitely established the infectiousness of the disease under Canadian conditions.

EFFECT OF INTRODUCING NEW SEED

These results ultimately supplied a full explanation of the failure to improve the crop in Nova Scotia by the selection method being used. A way was found in the meantime, however, to remedy the situation, that is, by the wholesale introduction from another district of sufficient healthy seed to replace practically all the old in one season. The new stock gave entire satisfaction for two years, but in the third season 3 per cent of leaf roll was not uncommon. As was to have been expected, some of the growers from lack of faith or excess of curiosity retained a little of their own seed. Thus patches containing 7, 11, 34 and 37 per cent of leaf roll were still found, in some cases beside the new seed. The result of this became finally evident in 1918. One grower after planting his main crop with new seed put in about 1 acre extra with the culls which were left from his old stock. One portion of the latter field developed as much as 13 per cent of leaf roll, while the main field had 1.5 per cent on the average. In the following year the percentage of disease on this farm ran up to 7 per cent although the worst seed was discarded.

Although in the case in which 13 per cent of leaf roll developed the disease tended to be peculiarly prevalent in one low-lying corner of the field, the general evidence with regard to the reappearance of the trouble is not in favor of the theory that infection persisted in the soil. There probably was enough of the old infected seed left to account for the second outbreak. This is in agreement with experience in Bermuda (3).

After the new seed had been grown in the district for four years the situation was again getting out of hand. There was practically no field above suspicion, and steps had to be taken again to circumvent the trouble as before by the general introduction of fresh stock from a practically non-infected district.

DISEASE-FREE DISTRICTS

The district used as a source of supply of healthy seed is situated only 75 miles from the affected area. The latter is in the fruit section of Nova Scotia, which is the warmest and probably the driest part of the province, surrounding the towns of Wolfville and Kentville. It was formerly believed that the local climate was mainly responsible for the disease situation. It is now doubtful if so much importance can be attached to this factor. In the first place comparatively healthy seed which has never showed more than two per cent of disease, has been maintained on one farm in the affected area without any care other than careful bin-selection. All around this farm from 5 to 50 per cent of leaf roll has been common. The seed is not noticeably resistant, for it has been freely infected both naturally and artificially. According to the record of the grower it has not been changed for many years. In the second place leaf roll has now appeared in a few farms in the previously disease-free district in considerable amounts. There had always been a trace present, generally not more than 1 per cent, but in 1917 up to 6 per cent was found in seed which one grower introduced from an unknown source. Thereafter leaf roll became more common, although all the infection must not be traced back to the case mentioned, for in some instances the growers were found to be raising other diseased varieties in 1919. Five Garnet fields were found containing from 3 to 3.5 per cent in 1918, and in 1919 six fields showed from 3 to 7.5 per cent of leaf roll. It is obvious in this case that what was previously attributed to climate seems to depend more on the restricted sources of infection which were present up until recent years. The conclusion is being reached that while climatic variations of such magnitude as exist between Charlottetown, Bermuda and Ottawa exert a pronounced influence on the relative prevalence of leaf roll, the smaller variations which occur within the Maritime Provinces cannot be held principally responsible for the local distribution of the disease. The extent to which sources of infection are present and the degree to which the disease spreads in the field seem to be dominant factors. The latter depends to some extent on varietal susceptibility, as well as on other factors the nature of which is unknown.

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(3) WORTLEY, E. J.: Potato leaf roll: its diagnosis and cause. Phytopath. **8**: 507–529, fig. 16. 1918.

A MACROSPORIUM FOOT-ROT OF TOMATO

J. Rosenbaum

With Four Figures in the Text

Numerous specimens of tomato plants were received in June and July, 1919, from Delaware, showing a disease the symptoms of which differed markedly from those of any known disease on tomatoes.

Symptoms. The symptoms as noted on these plants had a marked resemblance to black leg on potato stems. In figure 1 is shown a photograph of several naturally infected tomato stems, taken at the time these were received. Such stems, at the point where they emerge from the surface of the soil, first turn a dark brown. This brown rot or canker on the stem spreads deep into the tissues and extends above and below the surface of the soil. With age, the infected portion of the stem turns darker, the tissues shrivel up and may break, and the stem falls over at the original point of infection. Occasionally similar brown to black spots are found on other parts of the stem above ground. In such cases it often spreads a considerable distance from the point of infection, involving the entire stem and terminal blossoms.

In many cases the rot at the base of the plants produces a girdling effect. If this occurs when the tomato plants are still young, they throw out new roots above this point. Accompanying the girdling and stem cankers, the plants show varying degrees of wilting and discoloration of the leaves.

The disease has been found on tomato plants of all ages, both in the seed bed and in the field. Where the original infection takes place has not been determined.

The symptoms as outlined above suggest the possible use of "foot-rot" as a common name for this disease on tomatoes.

Losses. It is hard to estimate at this time how serious or how widespread the disease may prove to be. In a letter from one of the growers received June 30, 1919, referring to this trouble, he states, "On going through the fields recently fully 40 per cent of the plants were found to be affected with this disease."

Isolation of causal organism. Microscopic examination of diseased stems showed fungous mycelium within the affected tissues. No distinctive spores or fruiting bodies were found. Isolations were made on a variety of agar plates. In brief, the procedure followed was to wash the

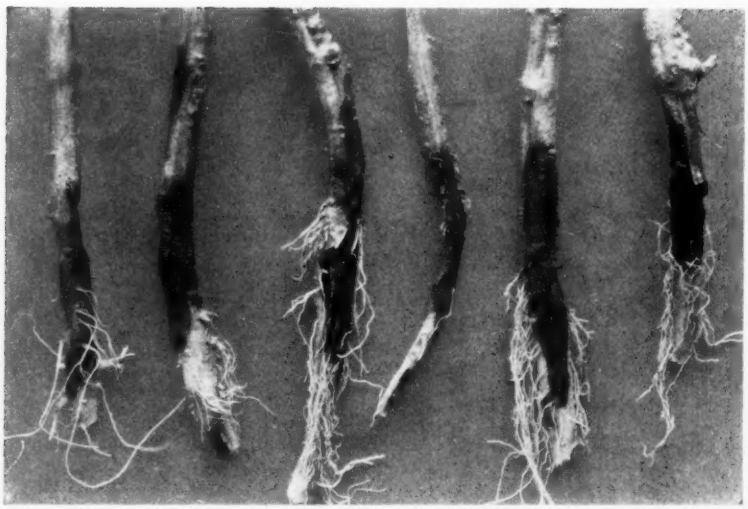

FIG. 1. LESIONS ON TOMATO STEMS DUE TO A MACROSPORIUM

Typical appearance of tomato stems affected with "foot-rot." Natural infection.

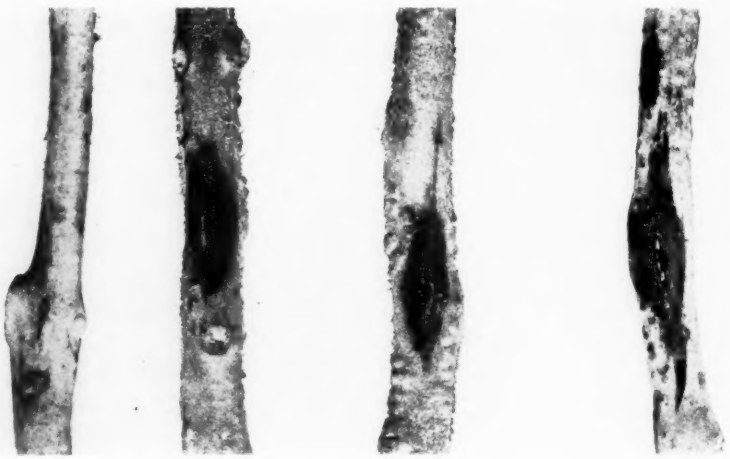

FIG. 2. LESIONS ON TOMATO STEMS ABOVE GROUND DUE TO INOCULATION WITH THE "FOOT-ROT" MACROSPORIUM

One check, as shown, remained healthy. Photograph taken eight days after inoculation.

affected stem, immerse in a 1:1000 bichloride of mercury solution for ten minutes, rinse in sterile distilled water, and make plantings from the inner tissues of the affected parts. Dilution plates on beef agar were also made. In the first series of isolations made, 71 per cent of the plantings gave a pure culture of the same fungus. The remainder of the plantings resulted in bacterial growth, or a mixture of bacteria and the same fungus as found in the majority of the colonies. During the progress of this work several series of isolations were made at various times from naturally infected stems. In all of these the same fungus was constantly associated with the lesions.

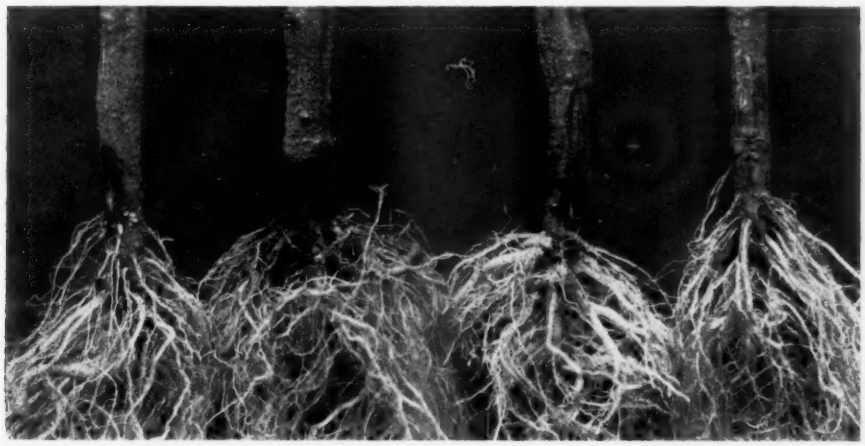

FIG. 3. LESIONS ON TOMATO STEMS BELOW SURFACE OF SOIL DUE TO INOCULATION WITH THE "FOOT-ROT" MACROSPORIUM

One check, as shown, remained healthy. Photograph taken eight days after inoculation.

Inoculations. Notwithstanding the predominance of the one fungus obtained from the isolations, it seemed advisable to test in addition, by artificial inoculations, the bacterial cultures obtained. Bouillon cultures of these bacteria were made and inoculated in a variety of ways on injured healthy plants in the greenhouse and in the field. The results in every case were negative.

With the fungus obtained from the isolations, a large number of inoculations were made, both on plants in the greenhouse and in the field. Two varieties of tomatoes were used, viz., Livingston's Globe and Earliana. The inoculations were made on the stems of healthy plants, both above ground and below, and near the surface of the soil. The procedure

followed was to cut the stem slightly with a flamed scalpel and insert in the wound a bit of fungus, from a pure culture grown on agar media. The inoculations made below the surface were either covered directly with soil or first covered with a piece of absorbent cotton moistened in sterile water, followed by soil; of the inoculations made above ground, part were left unprotected and the others were likewise covered with a piece of absorbent cotton moistened in water. Check plants were injured and treated in a similar manner, but without inserting the fungus into the cut stem.

The first series of inoculations were made July 3. When these were examined eight days later, the results obtained were so conclusive as to leave little, if any, doubt regarding the cause of this disease.

While the size and the resulting lesions varied in the field and in the greenhouse and on plants of different ages, all showed positive infection. Of the thirty-four plants inoculated, not a single one remained healthy. The checks, on the other hand, in every case remained unaffected. Figures 2 and 3 show the results of part of one series of inoculations. The photographs were taken eight days after the inoculations were made. In each case one check is shown.

Re-isolations. Re-isolations were made from a number of these plants. These resulted in a fungus growth similar to the one originally obtained from the naturally infected plants.

Identity of the causal fungus. The fungus causing the disease makes a vigorous mycelial growth on a variety of agar media. Neither on the host nor in culture does it produce spores readily. On account of its resemblance to other Macrosporium cultures, it was subjected to the treatment recommended by Kunkel[1] to obtain sporulation with *Macrosporium solani*. The treatment consists of wounding vigorously growing colonies of the fungus mycelium. With this treatment an abundant sporulation was obtained. The spores proved beyond a doubt that the fungus belongs to the genus Macrosporium. In figure 4 are shown camera lucida drawings of several typical spores of this fungus obtained in pure culture, and, for comparison, three other groups of Macrosporium spores. The latter were obtained from cultures of Macrosporium from potato leaves, from typical "nail-head" spots on tomato fruit, and from a rot on tomato fruit from New Jersey.

Examination of the literature failed to reveal any mention of a Macrosporium on tomatoes causing a disease with symptoms such as are described herein. In the Southern and Gulf States, where a Macrosporium on tomatoes causes serious damage and affects the leaves, stems, and fruit,

[1] Kunkel, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. & M. Brooklyn Bot. Garden Mem. **1**: 306–312. 1918.

the lesions produced on the stems are small "nail-head" spots, rarely, if ever, penetrating very deep into the tissues of the stem. The symptoms of the known Macrosporium diseases are therefore in marked contrast to those described here.

In view of these differences it was of interest to compare the Macrosporium obtained in this case with the others discussed in this paper, as shown in figure 4.

Comparisons[2] were made as regards (a) growth on media, (b) inoculations on various parts of the tomato plant,—stems, leaves, and fruit, and (c) measurements of the spores.

All of the Macrosporiums under discussion were grown on various agar media. The fungus isolated from typical "nail-head" spots could, with ease, be distinguished in each case from the other three Macrosporiums. The latter produced a growth which was characterized by a deep red or claret discoloration of the substratum of the media. The intensity of this color is dependent upon the alkalinity or acidity of the agar. The color is soluble in water and chloroform, but insoluble, or only slightly so, in ethyl alcohol and carbon bisulphide. It exhibits the properties of an indicator, being bright yellow when acidified and red when alkaline. It breaks down upon standing in an alkaline solution in the light.

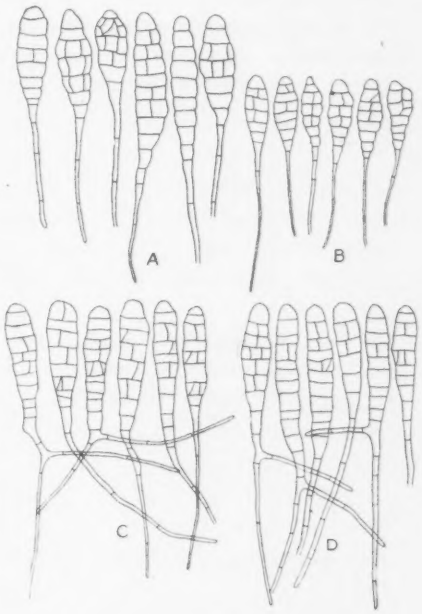

FIG. 4. MACROSPORIUM SPORES FROM TOMATOES AND POTATOES

Spores obtained in pure culture on hard potato agar. A. *M. solani*. Isolated from potato leaves from Maine.

B. *M. tomato*. Isolated from typical "nail-head" spots on tomatoes from Florida.

C. *M. solani*. Isolated from tomato plants affected with "foot rot" from Delaware.

D. *M. solani*. Isolated from potato seedlings affected with damping-off from Maine.

The Delaware fungus, while not identical in appearance on the various agar media with *Macrosporium solani* from potatoes, certainly resembles

[2] An account of studies made of Macrosporiums isolated from tomatoes from different sections of the United States was published in Phytopathology **10**: 7–22. 1920. In this article the spores marked "D" in figure 1 should be referred to as saprophytic Alternaria instead of a Macrosporium, as the spores are borne in chains.

TABLE 1

Inoculations with Macrosporium

SECTION OF PLANT INOCULATED	METHOD OF INOCULATION	CULTURE USED	RESULT OF INOCULATIONS	
			Infection	Character of lesion produced
Stems and leaves	Sprayed with suspensions of spores produced in pure culture	"Nail-head" Macrosporium from tomato fruit	+	"Nail-head" spots in abundance
		Delaware fungus from tomato stems	+	And occasional "nail-head" spot on leaves
		New Jersey Macrosporium from tomato fruit	+	
		Macrosporium solani from potato leaves	+	
Stems	Incisions made with a flamed scalpel and piece of fungus mycelium inserted in the cut	"Nail-head" Macrosporium from tomato fruit	−	
		Delaware fungus from tomato stems	+	Dark brown rot or canker. Girdling of stems
		New Jersey Macrosporium from tomato fruit	+	Similar to the ones produced by the Delaware fungus, but smaller and not as penetrating
		Macrosporium solani from potato leaves	+	
Fruit—Various stages of maturity	Sprayed with a suspension of spores produced in pure culture. Fruit not injured	"Nail-head" Macrosporium from tomato fruit	+	Typical "nail-head" spots
		Delaware fungus from tomato stems	−	
		New Jersey Macrosporium from tomato fruit	−	
		Macrosporium solani from potato leaves	−	
	Incision made with a flamed scalpel and piece of agar with pure growing culture inserted in cut	"Nail-head" Macrosporium from tomato fruit	+	Small, gray-black spot
		Delaware fungus from tomato stems	+	Large, firm, dark brown lesions, extending deep into the tissues
		New Jersey Macrosporium from tomato fruit	+	
		Macrosporium solani from potato leaves	+	

it. It should be stated that while the culture of *M. solani* which was used for comparison in this case produced a discoloration of the substratum, the writer has seen and worked with other cultures of *Macrosporium solani* which apparently do not produce any discoloration whatsoever. The Delaware fungus also agrees in cultural characteristics with the one from New Jersey.

In order to compare the pathogenicity of these Macrosporium cultures and the character of the lesions which they are able to produce, inoculations were made on the stems, leaves, and fruit with each form. A summary of the results is given in table 1. It shows the portion of the plant inoculated, the method of inoculation, and the results obtained in each case. The data presented again points to the fact that the Delaware stem Macrosporium agrees closely with *Macrosporium solani* from

TABLE 2

Spore measurements of Macrosporium

CULTURE	SPORE BODY, IN MICRONS						BODY OF SPORE, PLUS BEAK, IN MICRONS			AVERAGE NUMBER OF SEPTA
	Minimum length	Maximum length	Minimum width	Maximum width	Average length	Average width	Minimum length	Maximum length	Average length	
Macrosporium from tomato stems (Delaware)	56.7	98.7	14.7	23.1	**75.1**	**18.0**	157.5	350.7	**244.1**	**8**
Macrosporium solani from potato leaves (Maine)	29.4	111.3	8.4	23.1	**67.0**	**18.2**	71.4	247.8	**153.1**	**7**
Macrosporium from "nail-head" spots from tomato fruit (Florida)	29.4	69.3	10.5	23.1	**47.3**	**16.9**	54.6	279.3	**132.6**	**6**
Macrosporium from tomato fruit (New Jersey)	42.0	128.2	10.5	25.2	**79.0**	**20.1**	88.2	407.6	**288.5**	**8**

potato leaves and with Macrosporium from tomato fruit from New Jersey. It is different from the Macrosporium obtained from typical "nail-head" spots on the tomato fruit, both as regards its pathogenicity and the character of the lesion produced.

To determine further the identity of this Macrosporium, measurements of one hundred spores were made from each of the cultures. The spores were obtained in each case from potato agar plates of the same age and grown under the same conditions. A summary of these measurements is given in table 2.

The Macrosporium isolated from the Delaware tomato stems is much larger than the Macrosporium isolated from "nail-head" spots on tomato fruit. The differences in size between the others are not significant. Figure 4 shows the comparative size of the spores from each of these cul-

tures. It also shows that the terminal beak of the Delaware Macrosporium is often forked. This is a frequent characteristic of the spores of *M. solani* from potatoes, as is well illustrated by Rands.[3] Finally, the beak of the spores of the Delaware fungus is coarser than that of the ordinary "nail-head" Macrosporium, but similar to *Macrosporium solani* from potato.

Consideration of the facts brought out in these comparisons leaves on doubt that the Macrosporium isolated from Delaware tomato stems is different from the Macrosporium isolated from "nail-head" spots on tomato fruit, but similar to *M. solani* from potatoes. It should therefore be referred to as *M. solani* E. & M.

SUMMARY

1. A stem disease of tomato plants was found doing considerable damage in Delaware. The symptoms of the disease suggest the possible use of "foot-rot" as the common name for this trouble.

2. Numerous isolations from infected plants and from artificial inoculations in the field and greenhouse establish the fact that a fungus belonging to the genus Macrosporium is the causal organism.

3. Comparisons of this Macrosporium were made with *M. solani* from potatoes and with Macrosporium cultures isolated from a tomato fruit rot from New Jersey and from typical "nail-head" spots on tomatoes from Florida. These comparisons show that the Macrosporium discussed here differs from the one isolated from typical "nail-head" spots from Florida, in culture and morphology as well as in the fact that the former is unable to produce infection on uninjured green fruit. It resembles *M. solani* and the Macrosporium isolated from tomato fruit from New Jersey, in culture, in the kind of lesions produced when used for inoculation on fruit and stems, and in morphological characters. It should therefore be referred to as *Macrosporium solani* E. & M.

BUREAU OF PLANT INDUSTRY
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[3] Rands, R. D. Early blight of potato and related plants. Wis. Agr. Exp. Sta. Bul. 42: 1–48, fig. 4. 1917.

BRIEFER ARTICLES

A FROST INJURY OF POTATOES

H. G. MacMillan

With Plate XIII

A peculiar condition of the younger leaves of potato plants during the early growing season has been often noted, for which there has been no satisfactory explanation. The leaves were dotted with small irregular necrotic areas usually less than a millimeter in diameter, the majority of the spots being on the basal half of the leaf, along the midrib and near the petiole. The spots rarely appeared, if ever, after the plant reached fair size and the season was advanced. There was always the possibility that they might have been due to flea-beetle injury, but there was no hole, which would have been characteristic. No reasonable cause could be assigned for the occurrence of these spots.

During February potatoes were planted in pots in the greenhouse for experimental purposes. It was necessary to remove them on April 5, and they were stored in the laboratory basement over night with the intention of placing them out of doors in the sun during the succeeding days. The following morning the plants were set out a few minutes before ten o'clock; the day was warm, the sun bright, and the sky clear. The thermometer registered 39° F. The plants at this time were large, normal, and perfectly healthy in every respect. They had been exposed to no insect attacks or spray mixtures, and had been otherwise carefully handled. On April 16, the newer leaves showed marked symptoms of injury.

The plant shown in plate XIII is characteristic of both the field symptoms and the appearance of the potted plants. The larger leaves which are in focus appear healthy and normal, while the center leaves show the very small necrotic areas. The tips of the larger leaf blades are free from spotting, whereas the bases of the smallest leaf blades are unspotted. The areas are very small, irregular, dead and shrunken. Free-hand sections examined under the microscope showed these areas to be free from parasites, and dried and dead, while the tissue around them appeared healthy and normal. Moreover, many microscopic areas were revealed, not visible heretofore, which involved only a few cells, reaching from the tracheae to the surface through the palisade tissue. Apparently these smaller areas were around stomata, though the cell structure could not be

determined on account of the death of the tissue. DeVries, in his treatise on the anatomy of the potato plant, says stomata sometimes occur in the upper epidermis. The larger macroscopic areas extended through the leaf from one epidermis to the other.

The spots had every appearance of being caused by mechanical injury, such as frost. As the circumstances were well known under which the plants were set out on the morning of April 6, the data indicate that injury was caused by frost. The barometer stood at 25.04 inches; the temperature was 39° F.; the day was fair with a light wind from the east. The relative humidity, as recorded by a hygrothermograph calibrated by a sling psychrometer a few days previous, was 45 per cent. According to the Psychrometric Tables of the United States Weather Bureau (W. B. No. 235, p. 73, 1912), a depression of the wet bulb thermometer of 7.5° F. accompanies an air temperature of 39° F. and relative humidity of 45 per cent. This would give a temperature of 31.5° F. for the wet bulb thermometer.

The potato plants had been watered heavily the night before and placed in a warm basement. At that time the now expanded center leaves of plate XIII were very small stipuliform whorled leaflets, the tip ends of the terminal leaflets alone being expanded. All the leaves were turgid, and water probably filled all the stomatal openings. Exposure of the plants in dry air suddenly chilled the moist parts to a freezing temperature, resulting in the frost necrosis, not observable at the time, and only becoming apparent upon the extended growth of the leaves. The environment of the small whorled leaves in a light breeze would be comparable to the wet bulb of the sling psychrometer.

As noted at the beginning, this malady has been observed in the field. During early June warm nights, followed by very sudden cooling in the early morning, often occur. On potatoes a few inches high during the coolest nights these necrotic areas ultimately occurred. The time of appearance lagged so far behind the occurrence that the connection was missed, but in the light of the pot experiment the necrosis of the leaves must have been due to frost injury.

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PLATE XIII

A POTATO PLANT SHOWING FROST INJURY IN THE FORM OF SMALL, IRREGULAR, NECROTIC AREAS ON THE YOUNG, INNER LEAVES

MacMillan: Potato Frost Injury

DEVICE FOR GROWING LARGE PLANTS IN STERILE MEDIA

J. K. WILSON

WITH ONE FIGURE IN THE TEXT

Many methods have been suggested for growing plants for experimental purposes. These may be divided into two classes: Those which attempt to grow plants under sterile conditions, and those which do not. Of the former methods two have been more or less successful, depending upon the size of the plant to be grown and the duration of the experiment. With small plants, and in experiments of short duration, plants have been grown in suitable containers which have kept the entire plant from infection throughout the experiment. In other methods only that portion of the plant and its surroundings which were of importance in the special investigation have been kept under sterile conditions. In this paper a method is described that is of this latter type.

Investigators have found that it is sometimes necessary to grow plants in a medium from which all other forms of life are excluded, in order that a particular point to be investigated may not be interfered with by some other factor. Since this point usually has to do with the absorption of some particular substance, the contamination of parts other than the absorbing system may not be of any consequence. Edward and Barlow (1) grew the entire plant under sterile conditions. Garman and Didlake (2) started their plants under methods designed for sterile cultures, and as soon as the plants developed, the tops were allowed to grow in the open. Schulow (3) grew plants under a semi-sterile condition and reported good results. The method followed by any investigator is usually developed to meet his particular requirements.

The inability to use satisfactorily in our work any of the methods reported led us to try many ways to remedy this condition.

The most promising method for large plants, such as maize, and one yielding a fair degree of success, is a modification of the method used by Schulow. Briefly, his method consisted in germinating seeds that had been treated with bromine water for twenty minutes. The plantlets resulting were transferred bodily to the mouth of a small-necked container in which was a sterile nutrient solution. They were held in place by sterile cotton, and after becoming sufficiently large were wrapped with sterile cotton and allowed to grow. This kept the roots in sterile surroundings and the tops in the open. The main objections to this method were the difficulty in transferring the plantlets and the great chance for contamination in such procedure. By making certain changes in this

method the transferring could be done as satisfactorily as the transfer of an ordinary bacterial culture.

The above method as modified by us is as follows:

SEED STERILIZATION

Seeds are rendered sterile by the calcium hypochlorite method recommended by Wilson (4). After disinfection the seeds are planted on a sterile medium, from which, after germination, they are transferred to the permanent position. The solid medium for this germination is usually composed of the same ingredients as those used in the large container, from which the plant eventually draws its nutrients, with 1 to $1\frac{1}{2}$ per cent of agar. Agar and distilled water alone may be used, but the former is recommended, so that if contamination is present it may be detected before the plant is transplanted to its permanent position. This medium is made in sufficient quantity to meet all requirements, and is distributed into large test tubes.

Since the roots of most plants spread out in a lateral direction and thus make it difficult to transplant them quickly and conveniently, some method should be used which will direct the root growth in a vertical direction. To accomplish this there is placed in each test tube a short piece of glass tubing, 25 mm. by 50 mm. (see fig. 1, *e*). When the medium is distributed into the tubes, enough is put into each to cover all but about 15 mm. of the tubing. After sterilization of the medium the sterile seeds are dropped into it, where they germinate and produce plantlets for subsequent use. As they grow the roots penetrate the agar medium and pass down to the bottom of the tube. When the roots have thus developed the short piece of glass tubing, with its agar and plantlet, is lifted out and set into the mouth of the prepared flask. This is accomplished with sterile forceps.

PREPARATION OF FLASKS

A flask of any size with a neck that can be properly packed with cotton may be used. As much of the nutrient solution is put into the flask as can be sterilized therein, and a piece of cloth is stretched over the mouth. A hole just large enough to permit the insertion of a piece of 20 to 30 mm. tubing is cut through the cloth (see fig. 1, *c*). This tubing is just large enough to allow the plant and its tube to telescope into it (described above).

The lower end is slightly constricted to prevent the inner tube with the plant from going too far into the flask. Enough cotton is wrapped around the tube to hold it firmly in place when it is pushed into the mouth of

the flask. The upper end of the tubing should be about even with the top of the flask. The lower end should extend below the cloth and cotton so that when the flask is full of liquid the latter will just touch the tubing but not the packing. The flask is then covered with a large inverted beaker, which should be deep enough to leave some space above the mouth of the flask for the growing plant. In this way sterility can be maintained without much danger of subsequent infection.

Since there is need for more solution in the flask than can be sterilized in it, an extra amount is prepared, which may be poured into the flask through the opening that is left. If this opening seems small, a funnel may be sterilized with the flask, and after the latter is filled with the nutrient solution the funnel may be removed.

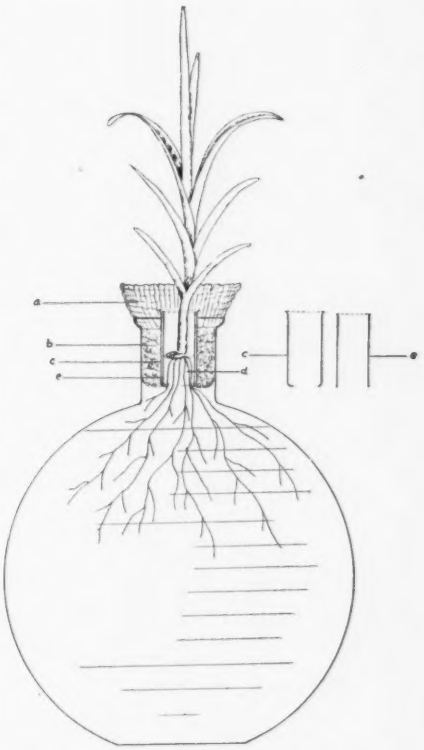

FIG. 1. DEVICE FOR GROWING LARGE PLANTS IN STERILE MEDIA

a, Cheese cloth; *b*, cotton wool; *c*, outside tube into which *e* slides; *d*, agar carried over from test tube with corn plant; *e*, tube in which seed germinates.

SETTING PLANTS

Before a plant is set into the mouth of the flask it should be inspected to note whether there is contamination. Plants transferred from the test tube to the flask need suffer no setback. If the plants are large enough when placed, they may be wrapped with sterile cotton and the beaker removed.

Maize, peas, and oats have been grown to maturity by this method. It should be noted that many kinds of nutrient solutions have been employed. The data presented in the following table were obtained from experiments in which there was no added organic material. The nutrient solution in all cases was of the same composition.

The table presented brings out very clearly what results may be expected from this method. In most cases about one hundred days elapsed before contamination was found. In the last series with maize, where

Sterility of nutrient solutions when crops were harvested

CROP	DATE STERILIZED	NO. OF FLASK																									
		1		2		3		4		5		6		7		8		9		10		11		12		13		14	
		Days to harvest																									
		Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility	Days	Sterility
Maize	June 21	28	+	43	+	56	+	70	−	98	−	85	−	91	−	98	−	108	−										
Oats	October 10	37	+	60	+	80	?	94	?	108	+	128	+	156	+	205		217											
Oats	February 12	43	+	78	+	99	+	99	+			106	+	123	−	129		141		150	−	150	−						
Maize	January 23	44	+	67	+	95	+			119	−	123	−	126	−	127	+			170	−								
Maize	December 18	60	+	73	+	76	+	87	+	96	+	101	−	108	+	122	+	133	+	133	+	150	+	150	+	150	+		
Maize	January 10	70	+	75	+	80	+	84	+	89	+	101	+	104	+	110	+	113	+	120	+	120	+	142	+	148	−	153	−

\+ = Sterile.
− = Not sterile.
? = Sterility questionable.

twelve flasks were harvested before the 148th day, no contamination was found. With maize, contamination may occur wherever the brace roots open up the cotton packing.

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GUMMING DISEASE OF SUGAR CANE IN PORTO RICO

J. MATZ

WITH ONE FIGURE IN THE TEXT

Gumming disease of sugar cane is known to have occurred in Australia (New South Wales and Queensland), Fiji Islands, Mauritius, Java, Borneo, New Guinea, Brazil, and very likely in Tucumán, Argentina. There is no record of it in the West Indies. The disease apparently does not occur in India.

In February, 1920, gumming of sugar cane was identified by the writer in two car loads of cane from the Trujillo Alto district, Porto Rico. The gummy exudation, varying in color from a lemon-yellow to that of honey, at the cut ends of the cane stalk, together with the bright-red vascular bundles in cane in the more advanced stages of the disease, are symptoms that agree with the gumming of cane of Australia as described by Cobb and studied by E. F. Smith. Cultures of the gum were made on potato agar, and the bacterial colonies as well as the transfers of these to potato plugs agree with those of *Bacterium vascularum* described by the last author. Masses of the bacterium in pure culture were smeared on needle-pricked surfaces of leaves of ten healthy, young "Otaheite" canes in an isolated greenhouse. After three months from inoculation all these plants showed, when cut, typical gummy exudation from the fibro-vascular bundles.

Immediately upon the recognition of the fact that gumming disease exists unmistakably in Porto Rico, a special survey was made of all the

principal sugar cane growing centers, and it was found that the disease is not wide-spread and that the exact locations of its occurrence are only in the Trujillo Alto district in an area of about fifteen kilometers. Specimens of the diseased cane were shown to men who have planted cane for years but all were unanimous in declaring it a new thing. One planter stated that he saw it to a very limited extent the previous year. The disease occurs in places where no new introductions of seed were made for many years past, and it was found in the oldest varieties grown on the island, namely, "*Otaheite*," "*Rayada*," "*Crystallina*" and "*Cavengerie*" or "*Colorada*." The "Otaheite" is the most severely infected, while the "Cavengerie" is the least infected variety. It is very likely that the disease

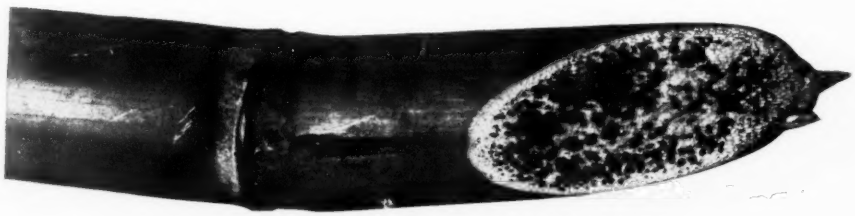

FIG. 1. GUMMING DISEASE OF SUGAR CANE

Gum drops exuding from the cut fibro-vascular bundles of "Otaheite" cane

has been here in insignificant proportions for some time in the past, but the unusually heavy rains at the beginning of the present year have most likely aided it in taking on the form of an epidemic in one district where "Otaheite" is still generally grown. Whether the disease will subside with the advent of normal rainfall remains to be seen. The only safe way to check it is through the planting of resistant varieties. So far the disease has not been observed in the variety "*Yellow Caledonia*." The fact that "*Rayada*" is susceptible makes it rather a difficult problem here, as this variety is very largely grown all over the island. "Cavengerie" shows decided resistance but it is not a very desirable variety here from the millers' point of view.

INSULAR EXPERIMENT STATION
RIO PIEDRAS, PORTO RICO

LITERATURE ON PLANT DISEASES[1]

COMPILED BY ALICE C. ATWOOD, AND FLORENCE P. SMITH, BIBLIOGRAPHICAL ASSISTANTS, BUREAU OF PLANT INDUSTRY

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